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Diversity and conservation of seasonal killifishes of the *Hypsolebias fulminantis* complex from a Caatinga semiarid upland plateau, São Francisco River basin, northeastern Brazil (Cyprinodontiformes, Aplocheilidae)

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Abstract

A high concentration of endemic species of seasonal killifishes has been recorded for a small area encompassing the highland plateaus associated with the upper section of the Carnaúba de Dentro River drainage and adjacent drainages of the middle section of the São Francisco River basin, northeastern Brazil. The present study is primarily directed to the taxonomy of the *H. fulminantis* species complex in this region, and describes habitat decline and extirpation of natural killifish populations recorded in field studies between 1993 and 2017. Both morphological characters and molecular species delimitation methods using single-locus models (GMYC and bPTP) support recognition of two closely related endemic species, *H. fulminantis* and *H. splendissimus* Costa, **sp. n.** The new species is distinguished from other congeners of the *H. fulminantis* complex by having a red pectoral fin in males, well-developed filamentous rays on the tips of the dorsal and anal fins in adult males, and the second proximal radial of the dorsal fin between the neural spines of the 8th and 9th vertebrae in males. Most recent field inventories indicated possible local extinction of populations of *H. fulminantis* and *H. splendissimus* in the studied area, but additional field studies should be made in other parts of the upper Carnaíba de Dentro River basin to evaluate the current conservation status of these species.

Introduction

In the last three decades, field studies of cynolebiine killifishes in temporary pools of the Caatinga, a semi-arid phytogeographical province of northeastern Brazil, have continuously revealed spectacular species diversity (e.g., Costa 2001, 2007, 2014; Costa et al. 2012, 2018a). Over 50 valid species of the two killifish genera occurring in the Caatinga, *Cynolebias* Steindachner, 1876 and *Hypsolebias* Costa, 2006, are endemic to the main river basins of the region, with a greater concentration of species in the São Francisco River basin (e.g., Costa et al. 2018b). Like other African and South American seasonal aplocheiloid killifishes, cynolebiine killifishes of the Caatinga are uniquely found in temporary pools formed during the rainy seasons, a specific kind of aquatic habitat that was not sampled by ichthyologists until the first studies of seasonal killifishes in the region (e.g., Costa and Brasil 1990, 1991, 1993). The life cycles of seasonal killifish are conditioned by irregular rainy seasons in the region mostly occurring between November and May, as well as by long dry periods, sometimes extending over a year, when species survive in resistant eggs buried in the pool substrate (Wourms 1972; Costa 1995).

In spite of the great morphological diversity exhibited by different endemic lineages of seasonal killifishes, several cryptic species have been recently recognised in the Caatinga using molecular species delimitation analyses

(e.g., Costa et al. 2012, 2014, 2018a). These studies have identified distinct cryptic species inhabiting the same drainage of the São Francisco River basin, showing that most seasonal killifish species exhibit a very restricted distribution range (Costa et al. 2012, 2018a). However, while field studies have been conducted to estimate killifish species diversity in the region, drastic anthropic modifications in seasonal killifish habitats of some Caatinga areas have caused extinction of several populations (Costa 2002, 2017; Costa et al. 2012, 2018a).

An uncommonly high concentration of endemic species of seasonal killifishes has been recorded for a small area encompassing the highland plateaus associated with the upper sections of the Carnaúba de Dentro and the Verde Pequeno river drainages, in the middle section of the São Francisco River basin (Costa and Brasil 1993; Costa et al. 1996; Costa and Nielsen 2004; Costa 2006a, 2014, 2017). This area is characterised by a series of plains located at slightly different altitudes, between 500 and 630 m above sea level (asl), separated from each other by an undulating relief and drained by temporary rivers and streams. Among the eight species reported for this area, two species, *Hypsolebias fulminantis* (Costa & Brasil, 1993) and *Hypsolebias carlettoi* (Costa & Nielsen, 2004) are members of a clade endemic to the Caatinga, which was named as J'-clade by Costa (2006b) and then diagnosed by the presence of a distinctive anteromedial process on the second hypobranchial, directed toward the second basibranchial (Costa 2006b: fig. 17c). Species of this clade are also unique among congeners by the highly contrasting colouration of the unpaired fins in males, consisting of intense bright blue marks over a red background, and the presence of intense red pigmentation on the trunk in males. The J'-clade also includes *H. shibattai* Nielsen, Martins, Araujo & Suzart, 2014, a species closely related to *H. fulminantis*, and a group known as the *Hypsolebias magnificus* species complex that comprises *H. gardneri* Costa, 2018, *H. hamadryades* Costa, 2018, *H. harmonicus* (Costa, 2010), *H. magnificus* (Costa & Brasil, 1991), and *H. picturatus* (Costa, 2000) (Costa 2007, 2010; Nielsen et al. 2014; Costa et al. 2018a). *Hypsolebias fulminantis* and *H. shibattai* form a consistent subclade, herein named the *H. fulminantis* species complex, easily diagnosed by the presence of narrow metallic blue lines parallel to the fin rays on all unpaired fins, in contrast to metallic blue dots or transverse blue stripes on the unpaired fins in other species of the group (Costa 2007). In addition, in species of this complex the opercular region and the anteroventral portion of the flank is intense yellow ochre in males, instead of pale golden as in other congeners of the J'-clade.

Hypsolebias fulminantis since its description in 1993, has become a popular aquarium fish due to the colouration exhibited by males. It was often collected by aquarists and amateur ichthyologists and consequently appears in numerous aquarium fish websites. However, field studies in the region have shown a sharp decline in natural populations (person. observation by WJEMC). In the past, *H. fulminantis* was frequently sampled around the town of Guanambi, in pools situated in the northeastern and southern parts of the town's periphery, at altitudes between about 525 and 555 m asl. However, during field studies in January 2010, after a severe environmental change in the region caused by the expansion of the Guanambi urban area, it was noted that all temporary pools sampled in previous years, inhabited by *H. fulminantis*, had been extirpated. On the other hand, new populations of seasonal killifishes were found just west from Guanambi, including a population with specimens similar to *H. fulminantis* but exhibiting some distinct morphological traits, suggesting that they may be a new species, which is here supported by molecular species delimitation methods. The objectives of this paper are to describe the new species and to provide a report on distribution and conservation of species of the *H. fulminantis* complex in the upper Carnaíba de Dentro River basin based on field studies made between February 1993 and March 2017.

Material and methods

Specimens

Methods for fish capture, euthanasia, fixation, and preservation in collections follow methods described by Costa et al. (2018a) for other seasonal killifishes of the Caatinga, which were approved by CEUA-CCS-UFRJ (Ethics Committee for Animal Use of Federal University of Rio de Janeiro; permit number: 065/18). Collections were made with permits provided by ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade; permit numbers: 34270-4, 20618-1, 57099-1). Preserved specimens listed in this paper are deposited in the ichthyological collections of: Museu de Zoologia, Universidade de São Paulo, São Paulo (MZUSP), and Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro (UFRJ). In lists of material, the abbreviation C&S indicates specimens prepared for osteological analysis and preserved in glycerine (see below), and DNA indicates specimens fixed and preserved in 98 % ethanol. List of specimens used in the molecular analysis and their respective GenBank accession numbers appears in Table 1. Comparative material is listed in Costa (2007, 2010) and Costa et al. (2018a).

Morphological data

Descriptions of colouration in living fish were based on photographs of both sides of individuals. Photographs were taken in small aquaria about 24 hours or less after collections. Additional direct observations were made with fish in small transparent plastic bottles just after collection. Measurements and counts follow Costa (1988). Measurements are presented as percentages of standard length (SL), except for those related to head morphology, which are expressed as percentages of head length. Measurements were made only in specimens fixed in 10 %

Table 1. List of specimens used in the molecular analysis, with their respective catalog numbers, coordinates of the collecting site, and GenBank accession numbers for cytb sequences. Asterisk indicates sequences not published previously.

Species	Catalog number	Coordinates	Cytb
Hypsolebias carlettoi	UFRJ 6780.1	14°13'42"S, 42°55'12"W	MH909076*
Hypsolebias carlettoi	UFRJ 6780.2	14°13'42"S, 42°55'12"W	MH048856
Hypsolebias carlettoi	UFRJ 6780.3	14°13'42"S, 42°55'12"W	MH909078*
Hypsolebias carlettoi	UFRJ 6780.4	14°13'42"S, 42°55'12"W	MH909079*
Hypsolebias fulminantis	UFRJ 6726.1	14°12'21"S, 42°45'42"W	MH048854
Hypsolebias fulminantis	UFRJ 6726.2	14°12'21"S, 42°45'42"W	MH909075*
Hypsolebias hellneri	UFRJ 6700.4	15°04'49"S, 44°04'40"W	MH909072*
Hypsolebias splendissimus	UFRJ 6778.1	14°12'54"S, 42°50'22"W	MH909080*
Hypsolebias splendissimus	UFRJ 6778.2	14°12'54"S, 42°50'22"W	MH909081*
Hypsolebias splendissimus	UFRJ 6778.3	14°12'54"S, 42°50'22"W	MH909082*
Hypsolebias splendissimus	UFRJ 6778.4	14°12'54"S, 42°50'22"W	MH909083*
Hypsolebias picturatus	UFRJ 6708.1	11°28'03"S, 43°17'10"W	MH048868

formalin for a period of 10 days, and then transferred to 70 % ethanol; specimens fixed in 98 % ethanol, and consequently having slightly deformed body by dehydration, were not measured. Fin-ray counts include all elements. At least four specimens of each species, two males and two females, were cleared and stained for osteological examination using Taylor and Van Dyke's (1985) protocol. Terminology for osteological structures followed Costa (2006b), for frontal squamation Hoedeman (1958), and for cephalic neuromast series Costa (2001). Meristic data were taken from all available specimens, except osteological characters that were taken only from cleared and stained (C&S) specimens.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from muscle tissue of the right side of the caudal peduncle using the DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's instructions. A fragment of the mitochondrial DNA gene cytochrome b (cytb) was amplified using the primers L14724 and H15149 (Kocher et al. 1989; Meyer et al. 1990). Polymerase chain reaction (PCR) was performed in 15 µl reaction mixtures containing 5× Green GoTaq Reaction Buffer (Promega), 3.2 mM $MgCl_2$, 1 µM of each primer, 75 ng of total genomic DNA, 0.2 mM of each dNTP and 1 U of Taq polymerase. The thermocycling profile was: (1) 1 cycle of 4 minutes at 94 °C; (2) 35 cycles of 1 minute at 92 °C, 1 minute at 44–54 °C and 1 minute at 72 °C; and (3) 1 cycle of 4 minutes at 72 °C. In all PCR reactions, negative controls without DNA were used to check for contaminations. Amplified PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega). Sequencing reactions were made using the BigDye Terminator Cycle Sequencing Mix (Applied Biosystems). Cycle sequencing reactions were performed in 10 µl reaction volumes containing 1 µl BigDye 2.5, 1.55 µl 5× sequencing buffer (Applied Biosystems), 2 µl of the amplified products (10–40ng), and 2 µl primer. The thermocycling profile was: (1) 35 cycles of 10 seconds at 96 °C, 5 seconds at 54 °C and 4 minutes at 60 °C. The sequencing reactions were purified and denatured and the samples were run on an ABI 3130 Genetic Analyzer. Sequences were edited using MEGA 6 (Tamura et al. 2013) and aligned using ClustalW (Chenna et al. 2003); alignments were subsequently translated into amino acids residues to check premature stop codons or indels. List of specimens used in the molecular analysis and their respective GenBank accession numbers appear in Table 1.

Phylogenetic analysis and species delimitation

Analyses were conducted with a short cytb fragment (416 bp) that has been efficiently used for delimitating cryptic species of different aplocheiloid killifish groups (Sonnenberg 2007; Van der Zee and Sonnenberg 2011; Costa et al. 2012, 2014, 2018a). Terminal taxa were 10 specimens of the three species of the J'-clade endemic to the upper Carnaíba de Dentro River drainage; out-groups comprised one species of the *H. magnificus* complex (*H. picturatus* (Costa, 2000)), and *H. hellneri* (Berkenkamp, 1993), the sister group of the J'-clade (Costa et al. 2017), which was used to root the phylogeny. The best-fit model of sequence evolution was calculated by jModelTest 2.1.7 (Darriba et al. 2012), which indicated the general-time reversible model with a gamma frequency distribution of categories among sites (GTR + G). Bayesian reconstruction was performed with BEAST v.1.8 (Drummond et al. 2012), using an uncorrelated relaxed lognormal model and other parameters set as default; the MCMC length was 30,000,000 runs with sampling every 1,000 runs. The quality of the MCMC chains was evaluated in Tracer 1.5 (Rambaut, et al. 2013); a 25% burn-in was removed and the final tree was obtained using TreeAnnotator v.1.5 from BEAST v.1.8 package; support values of the Bayesian inference (BI) analysis were calculated by posterior probability. Two different single-locus models for species delimitation were used: the Generalized Mixed Yule-Coalescent (GMYC), independently applying single and multiple-threshold (Fujisawa and Barraclough 2013), and the Bayesian implementation of Poisson Tree Process (bPTP), using both Maximum likelihood and Bayesian solutions (Zhang et al. 2013), with 500,000 Markov chain Monte Carlo (MCMC) generations, thin-

ning set to 100 and a burn-in of 25% initial samples. All analyses were carried on the Exelixis Lab's web server (GMYC at http://species.h-its.org/gmyc/; bPTP at http://species.h-its.org/ptp/).

Conservation data

Descriptions of field data relative to habitat conservation were made during collecting trips between 1994 and 2017 (February 1994, February 1999, May 1999, January 2002, January 2005, May 2009, January 2010, January 2017, and April 2017).

Results

The phylogenetic analysis generated a tree with most branches supported by highest posterior probability values (Fig. 1). This analysis strongly supports *H. carlettoi* as being more closely related to species of the *H. fulminantis* complex than to *H. picturatus*, which is a member of the *H. magnificus* complex (Costa et al. 2018a). All methods of species delimitation yielded identical results, supporting *H. fulminantis* and the population from the pool just west of Guanambi as a distinct species, which is described below.

Hypsolebias splendissimus Costa sp. n.

http://zoobank.org/77B46AC3-448F-4CBF-94E0-487D303E36A4

Figs 2–3, Table 2

Holotype. UFRJ 6909, male, 42.7 mm SL; Brazil: State of Bahia state: Municipality of Guanambi: temporary pool close to road BR-030, about 1.5 km W from the confluence between the Poço do Magro River and the Carnaíba de Dentro River, São Francisco River basin, and about 3 km W of the town of Guanambi, 14°12'54" S 42°50'22" W, altitude about 505 m asl; W. J. E. M. Costa et al., 30 January 2010.

Paratypes. UFRJ 6779, 1 male, 43.3 mm SL, 2 females, 28.5–30.2 mm SL; UFRJ 6910, 1 male, 42.7 mm SL, 3 females, 26.7–30.5 mm SL (C&S); UFRJ 6778, 2 males, 33.7–36.2 mm SL, 6 females, 28.4 – 29.5 mm SL (DNA); collected with holotype.

Diagnosis. *Hypsolebias splendissimus* differs from *H. fulminantis* and *H. shibattai* by having: pectoral fin red in males (vs. hyaline in *H. fulminantis* and *H. shibattai*), well-developed filamentous rays on the tips of the dorsal and anal fins in adult males (vs. filamentous rays absent or rudimentary, poorly visible), and the second proximal radial of the dorsal fin between the neural spines of the 8th and 9th vertebrae in males (vs. between the neural spines of the 6th and 7th vertebrae). Also distinguished from *H. shibattai* by having the dorsal-fin origin posterior to the anal-fin origin in males (vs. anterior), distinctive red bars restricted to the anterior portion of the flank males (vs. extending over the whole flank), and absence of contact organs on the pectoral fin in males (vs. present).

Table 2. Morphometric data of *Hypsolebias splendissimus*.

	Holotype	Paratypes	
	Male	Males (2)	Females (5)
Standard length (mm)	42.7	42.9–43.3	27.0–30.5
Percent of standard length			
Body depth	36.0	35.0–36.8	35.8–38.8
Caudal peduncle depth	16.1	15.5–16.0	14.9–15.6
Pre-dorsal length	45.2	47.3–48.0	58.0–62.3
Pre-pelvic length	42.2	42.7–43.6	49.9–52.0
Length of dorsal-fin base	42.9	39.6–40.1	24.3–28.8
Length of anal-fin base	42.1	40.0–43.8	23.2–26.7
Caudal-fin length	40.2	40.0–41.3	34.7–37.8
Pectoral-fin length	28.2	28.8–29.1	24.1–25.7
Pelvic-fin length	10.7	10.4–11.6	10.6–12.2
Head length	27.1	26.5–27.7	28.3–31.1
Percent of head length			
Head depth	109.9	111.9–114.3	102.5–97.6
Head width	63.9	67.1–68.7	65.2–74.4
Snout length	14.7	12.9–15.5	13.8–14.8
Lower jaw length	19.9	17.7–18.0	14.9–16.5
Eye diameter	28.4	29.3–32.0	31.3–37.1

Description. Morphometric data appear in Table 2. Body relatively deep, compressed. Greatest body depth at vertical just anterior to pelvic-fin base. Dorsal and ventral profiles of head and trunk slightly convex, approximately straight on caudal peduncle. Head narrow, sub-triangular in lateral view. Jaws short, teeth numerous, conical, irregularly arranged; outer teeth hypertrophied, inner teeth small and numerous. Vomerine teeth absent. Gill-rakers on first branchial arch 2 + 10–11, gill-rakers short, straight, without denticles.

Dorsal and anal fins pointed in males, with two or three filaments on tip, rounded, without filaments, in females. Caudal fin rounded. Pectoral fin sub-lanceolate, posterior tip reaching vertical between base of 5th and 7th anal-fin rays in males, reaching between anus and urogenital papilla in females. Pelvic fin small, tip reaching base of 3rd anal-fin ray in males, reaching between urogenital papilla and anal-fin origin in females; pelvic-fin bases medially united. Dorsal-fin origin on vertical between base of 2nd and 4th anal-fin rays in males, between base of 4th and 6th anal-fin rays in females. Dorsal-fin rays 19–22 in males, 15–16 in females; anal-fin rays 21 in males, 18–19 in females; caudal-fin rays 23–24; pectoral-fin rays 12–13; pelvic-fin rays 6. No contact organs on fins. Second proximal radial of dorsal fin between neural spines of 8th and 9th vertebrae in males, between neural spines of 11th and 12th vertebrae in females; first proximal radial of anal fin between pleural ribs of 8th and 9th vertebrae in males, between pleural ribs of 9th and 10th vertebrae in females; total vertebrae 26–27.

Scales small, cycloid. Body and head entirely scaled, except anterior ventral surface of head. Body squamation

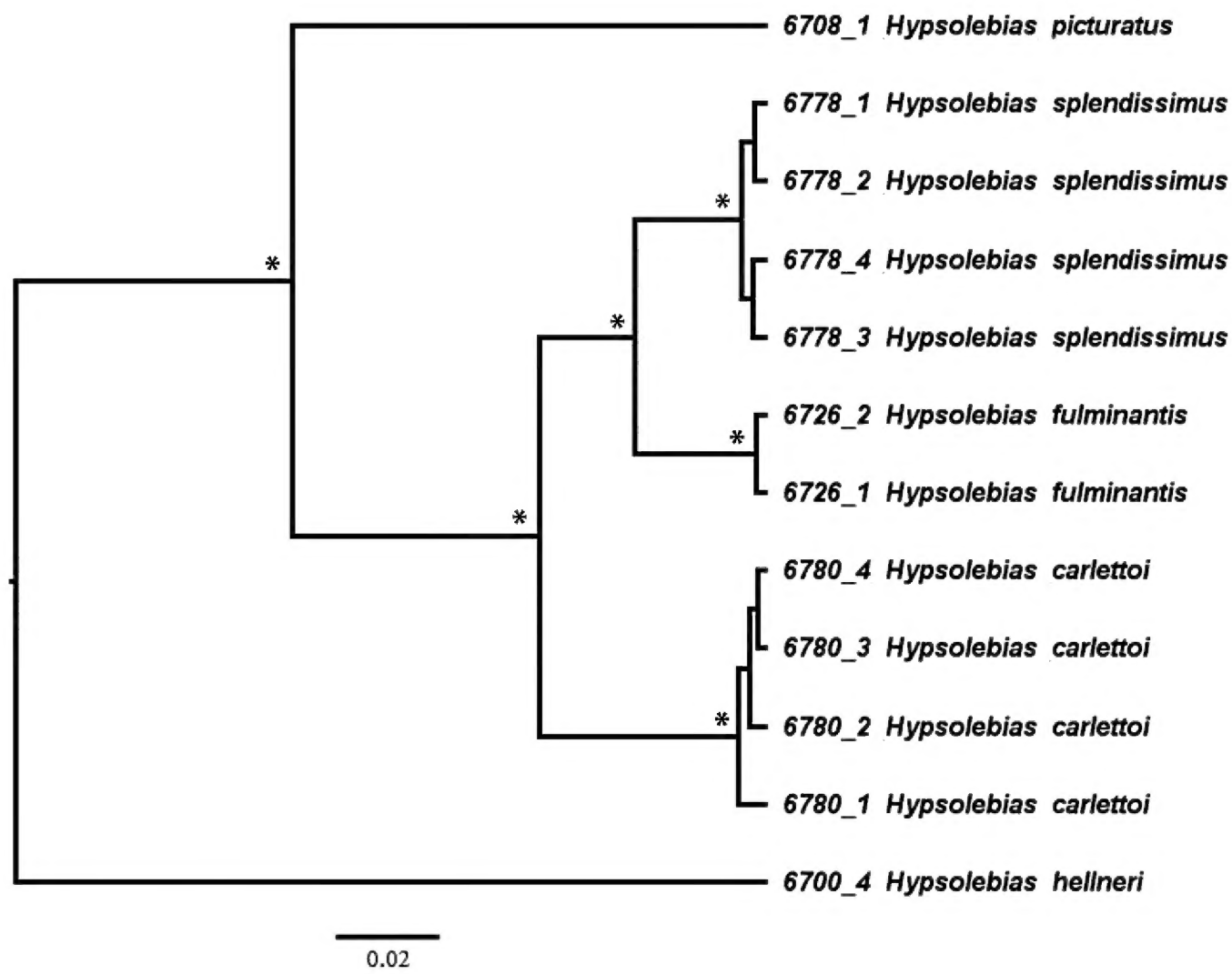

Figure 1. Bayesian phylogeny used to delimit species endemic to the upper Carnaíba de Dentro River drainage, inferred by using sequences of the mitochondrial gene cytochrome b, 416 bp. Posterior probability values below 95% are not depicted; asterisk above nodes represents maximum value of posterior probability (100 %); numbers before species names are catalogue numbers for voucher specimens.

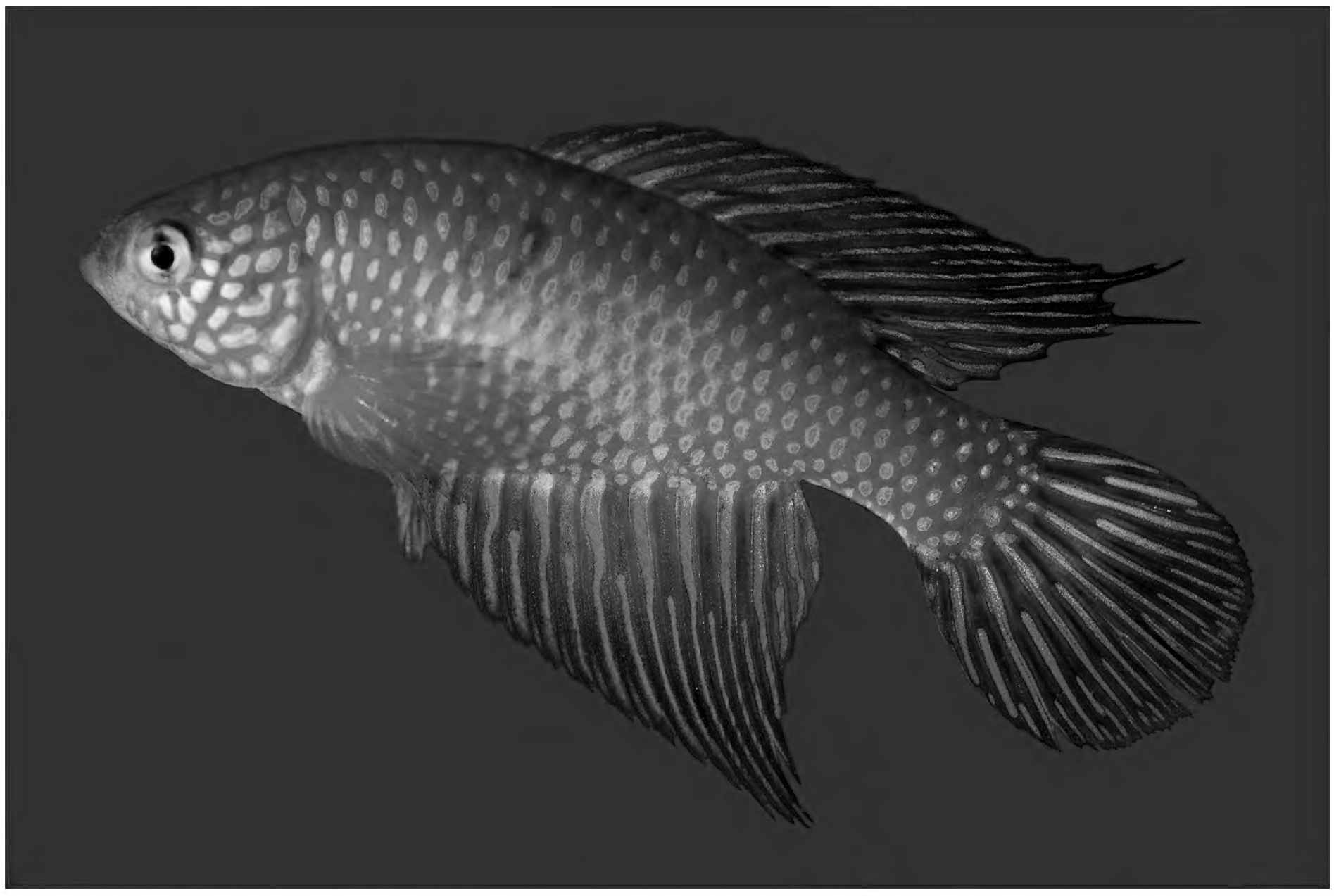

Figure 2. *Hypsolebias splendissimus* Costa sp. n., live holotype, UFRJ 6909, male, 42.7 mm SL. Photograph by W.J.E.M. Costa.

Figure 3. *Hypsolebias splendissimus* Costa sp. n., live paratype, UFRJ 6779, female, 28.5 mm SL. Photograph by W.J.E.M. Costa.

extending over anterior 20 % of caudal-fin base; scales slightly extending on middle part of anal-fin base in males. Frontal scales E-patterned. Longitudinal series of scales 25–26; transverse series of scales 11; scale rows around caudal peduncle 12. One prominent contact organ on each flank scale in males. Cephalic neuromasts: supraorbital 12–16; parietal 2; anterior rostral 1, posterior rostral 1; infraorbital 3 + 22–24; preorbital 3–4; otic 2, post-otic 2; supratemporal 1; median opercular 1, ventral opercular 1–2; pre-opercular 15–16; mandibular 10–13; lateral mandibular 4, paramandibular 1.

Colouration in life. ***Males***. Flank intense red to pink on middle portion and metallic yellow ochre on anteroventral part; small, vertically elongated bright blue spot on centre of each scale; central portion of flank often with distinctive red bars, alternating with faint green bars, sometimes inconspicuous. Dorsum pale yellowish brown, venter yellowish white. Side of head metallic light blue, with red scale margins on dorsal portion and intense metallic yellow ochre on opercular, post-orbital and infra-orbital regions; snout and jaws light grey. Iris light yellow to pale orange, with dark brown bar through orbit centre. Unpaired fins red, with alternating short and long metallic blue lines to greenish golden lines, depending on angle of light incidence, parallel to fin rays; dorsal and anal fin filaments dark grey to black. Pelvic fin red with light blue rays. Pectoral fin red.

Females. Flank light brownish grey, to yellowish grey on dorsal portion and pale golden on anteroventral portion; two or three oval black spots on antero-central portion of flank; smaller specimens, about 28 mm SL or less, with dark grey bars, often interrupted; larger specimens above 28 mm SL, with dark grey spots on whole flank, often arranged in vertical rows, becoming dark grey to black around antero-central spots. Dorsum yellowish grey, venter white. Side of head yellowish grey, pale greenish golden on opercular and post-orbital regions; jaws light grey. Iris light yellow to pale orange, with dark brown bar through orbit centre. Fins yellowish hyaline.

Colouration in alcohol. Trunk and head pale brown, with faint grey bars on anterior portion of flank in males, and grey spots on flank in females. Fins grey in males, hyaline in females. No vestige of red pigmentation and blue iridescent marks.

Distribution, habitat and conservation. *Hypsolebias splendissimus* is known from a single collection at the type locality, a temporary pool in a flat plains area about 1.5 km W from the confluence between the Poço do Magro and Carnaíba de Dentro rivers, middle São Francisco River basin, Bahia, Brazil (14°12'54" S 42°50'22" W, altitude about 505 m asl; Fig. 4). At the time of the type series collection (30 January 2010) the pool was about 100 m long and 30 m wide, with a maximum depth of about 0.5 m. All individuals of *H. splendissimus* were concentrated in one part of the pool, near its margin, in an area about 100 m^2, that was densely populated by shrubs and aquatic plants, forming a distinctive shaded habitat. This site was visited again in January 2017, but the entire pool had been drained and landfilled by bulldozers and the new species was not found again.

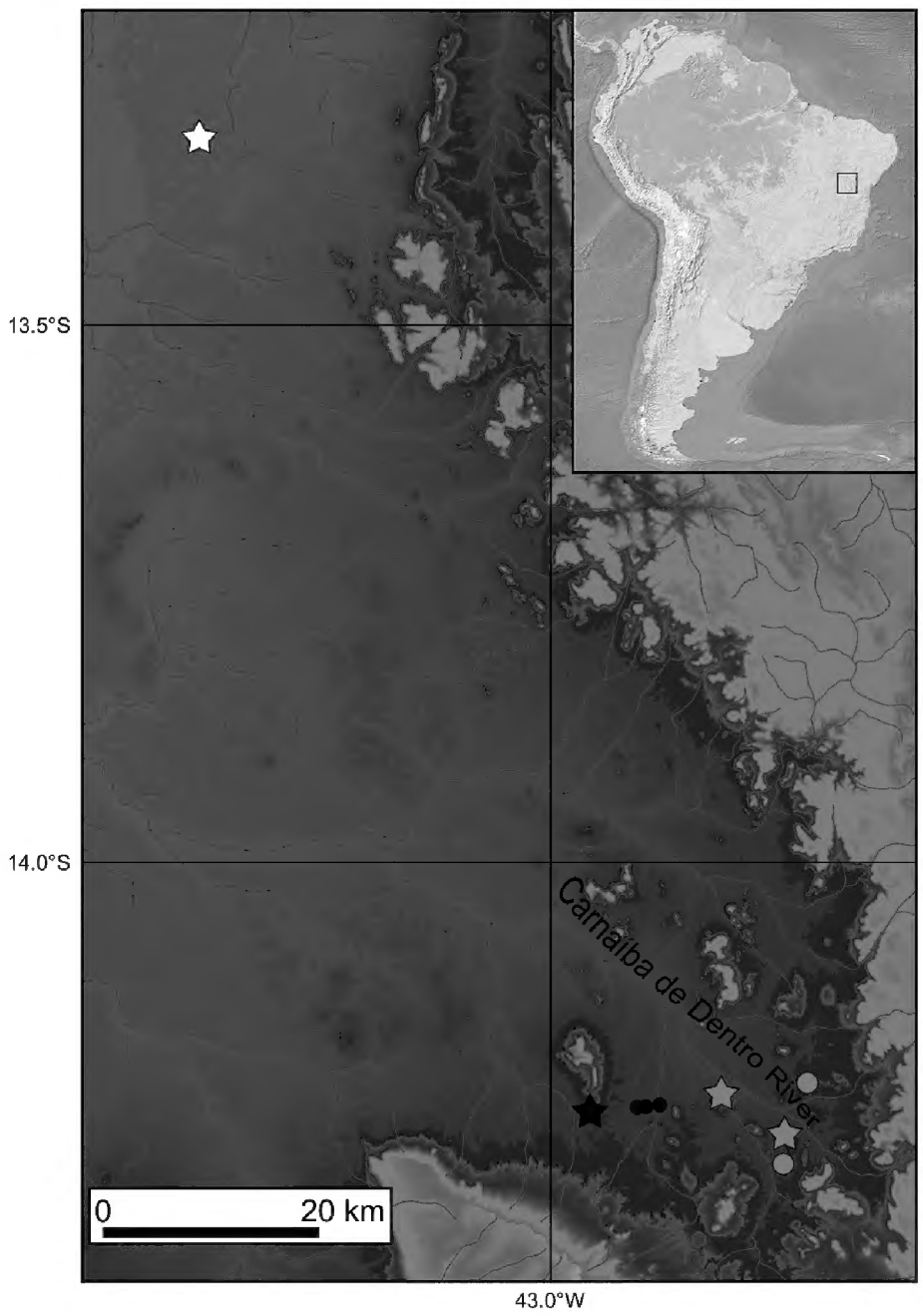

Figure 4. Geographical distribution of species of the *Hypsolebias* J'-clade in the upper Carnaíba de Dentro River drainage (yellow, *H. fulminantis*; red, *H. splendissimus*; black, *H. carlettoi*) and *H. shibattai* (white); stars indicate type localities.

Etymology. From the Latin *splendissimus* (very splendid), an allusion to the bright colours in males of the new species, which is among the most colourful South American aplocheiloid killifishes.

Hypsolebias fulminantis (Costa & Brasil, 1993)

Figs. 5, 6

Cynolebias fulminantis Costa & Brasil, 1993: 194 (type locality: swamp near Guanambi [road BR-122], Estado da Bahia, northeastern Brazil [14°15'16"S, 42°46'56"W, altitude about 555 m]; MZUSP 43674).

Diagnosis. *Hypsolebias fulminantis* is a member of the *H. fulminantis* complex, differing from *H. splendissimus* by: the presence of hyaline pectoral fins in males (vs. red), presence of rudimentary or absence of filamentous rays on the tips of the dorsal and anal fins in adult males (vs. well-developed filamentous rays present), and the second proximal radial of the dorsal fin situated between the neural spines of the 6th and 7th vertebrae in males (vs. between the neural spines of the 8th and 9th vertebrae); and from *H. shibattai* by having the dorsal-fin origin posterior to the anal-fin origin in males (vs. anterior); distinctive red bars restricted to the anterior portion of the flank males (vs. extending over the whole flank); and absence of contact organs on the pectoral fin in males (vs. present).

Distribution, habitat and conservation. *Hypsolebias fulminantis* has been recorded from several localities in the upper Carnaíba de Dentro River basin, close to the town of Guanambi, in altitudes between 525–555 m asl (Fig. 4). These pools were shallow, maximum depth about 0.5 m, with their surface between about 15 and 300 m^2, and always densely occupied by aquatic plants, except in parts where recent anthropic modifications were recorded. *Hypsolebias fulminantis* was always found close to the pool margins, in shadier places. In 1994, this kind of habitat was abundant in the region, but some decline was already recorded in 1999 (Costa 2002). Previously unsampled pools inhabited by *H. fulminantis* were found in January 2002 and January 2005. After an intense expansion of the urban area, field studies in May 2009, January 2010, and January and April 2017 failed to find any specimen of *H. fulminantis* in the region.

Remarks. For a full description, see Costa (2007) based on types and other specimens collected in the type locality area.

Material examined. Brazil: State of Bahia: Municipality of Guanambi: São Francisco River basin, upper Carnaíba de Dentro River drainage: MZUSP 43674, holotype, male, 38.9 mm SL; MZUSP 43675, 2 paratypes; UFRJ 685, 2 paratypes; UFRJ 686, 3 paratypes; Guanambi, road BR-122, 14°15'16"S, 42°46'56"W, altitude about 555 m; G. C. Brasil, 1 Jan. 1992. – UFRJ 6068, 6; UFRJ 6069, 2; UFRJ 6726, 3; Guanambi, road BR-030, 14°12'21"S, 42°45'42"W, altitude about 545 m; W. J. E. M. Costa et al., 13 Jan. 2005. – UFRJ 4802, 1; temporary pool about 4.5 km S from Guanambi, Rio road BR-122,14°16'49"S, 42°47'01"W, altitude about 525 m; W. J. E. M. Costa et al., 11 Feb. 1999. – UFRJ 4847, 2; same locality as UFRJ 4802; W. J. E. M. Costa et al., 4 May 1999. – UFRJ 3809, 6; UFRJ 5864, 4 (C&S); temporary pool 4.5 km S from Guanambi; A. L. F. Cyrino et al., 27 Jan. 1996.

Discussion

Hypsolebias splendissimus is presently known from a single locality just 8 km west from the geographical area inhabited by *H. fulminantis* (Fig. 4). Their distribution areas are situated in neighbouring sub-drainages of the upper section of the Carnaíba de Dentro River drainage, at slightly different altitudes, about 505 m asl at the type locality of *H. splendissimus* and between 525 and 555 m asl at the localities from where *H. fulminantis* has been recorded. Despite their geographical proximity, both morphological characters (see diagnosis above) and molecular data (Fig. 1) support recognition of them as two different species.

Field studies in the Caatinga have shown that *H. carlettoi* is also endemic to the upper Carnaíba de Dentro River drainage, but it was never found in sympatry with

Figure 5. *Hypsolebias fulminantis*, UFRJ 4847, male, 44.0 mm SL. Photograph by W.J.E.M. Costa.

H. fulminantis or *H. splendissimus*. Its distribution range is situated in a different subdrainage of the Upper Carnaíba de Dentro River drainage, the Mutula River subdrainage, and is separated by a distance of about 7 km from the type locality of *H. splendissimus* and about 15 km from the recorded geographical range of *H. fulminantis* (Fig. 4). In morphological analyses, *H. carlettoi* was considered to be more closely related to species of the *H. magnificus* complex than to *H. fulminantis* by exhibiting a red pectoral fin in males, contrasting with the hyaline pectoral fin in males of *H. fulminantis* and *H. shibattai*, which would be a plesiomorphic condition for cynolebiine killifishes (Costa 2006b, 2007). However, molecular analyses indicated that *H. carlettoi* is more closely related to *H. fulminantis* than to species of the *H. magnificus* complex (Costa et al. 2018a), a finding that is also corroborated here (Fig. 1), refuting the presence of red pectoral fins as an unambiguous synapomorphy for a subclade of the J'-clade including only *H. carlettoi* and species of the *H. magnificus* complex. The presence of a red male pectoral fin only in *H. splendissimus* among species of the *H. fulminantis* complex may be tentatively interpreted as a plesiomorphic condition for the J'-clade lost in *H. fulminantis* and *H. shibattai*, in which the pectoral fin is always hyaline in contrast to red as in the remaining congeners of the J'-clade. This colour pattern character thus suggests that *H. fulminantis* is more closely related to *H. shibattai* that is endemic to a distant area, about 115 km from the area herein studied (Fig. 4), than to *H. splendissimus* that inhabits a neighbouring area. However, molecular data for *H. shibattai* are not available, making interpretations about relationships among these three species weak.

The present study reports an accentuated decline in seasonal killifish habitats in the upper Carnaíba de Dentro River drainage around the town of Guanambi, possibly causing local extinction of *H. fulminantis* and *H. splendissimus*. However, most parts of the Carnaíba de Dentro River drainage are still not easily accessible and field studies to detect the occurrence of seasonal killifishes have never been conducted. So at this time it is not possible to evaluate the conservation status of *H. fulminantis* and *H. splendissimus*. On the other hand, satellite images indicate that these unsampled areas are extensively modified for agriculture, an environmental impact that usually has negatively affected seasonal killifish habitats (Costa 2002). Species of the J'-clade are particularly vulnerable, since they are only found in shaded parts of the pool and consequently habitat deforestation results in quick extirpation of species, even when open vegetation parts of the pool are not destroyed (Costa et al. 2018a). This study indicates the urgency to conduct additional inventories of the seasonal killifishes found in the temporary pools of the Carnaíba de Dentro River drainage to accurately establish their distribution and conservation status.

Figure 6. *Hypsolebias fulminantis*, UFRJ 4847, female, 34.0 mm SL. Photograph by W.J.E.M. Costa.

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